# HETERODIMERIC PEPTIDE REAGENTS AND METHODS

## STATEMENT OF GOVERNMENT INTEREST

**[0001]** This invention was made with government support under CA163059 and CA189291, awarded by the National Institutes of Health. The government has certain rights in the invention.

## INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

**[0002]** Incorporated by reference in its entirety is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: 5,923 bytes ASCII (Text) file named "52934A_SeqListing.txt," created on May 15, 2019.

## FIELD

**[0003]** The disclosure is directed to heterodimeric peptide reagents as well as methods for detecting and targeting esophageal adenocarcinoma cells using the heterodimer peptide reagents.

## BACKGROUND

**[0004]** Esophageal adenocarcinoma (EAC) is a deadly cancer that is rising rapidly in incidence, and is associated with a poor prognosis and low five-year survival [Torre et al., Cancer Epidemiol. Biomarkers Prevent. 25: 16-27 (2016)]. Barrett's esophagus (BE) represents a metaplastic transformation of squamous into specialized columnar epithelium in response to long-standing acid and bile reflux [Spechler et al., N. Engl. J. Med. 371:836-45 (2014)]. BE is becoming more common as a result of an increasing prevalence in obesity [Whiteman et al., Gut 57(2):173-80 (2008); electronically published, Oct. 11, 2007]. High-grade dysplasia (HGD) is a pre-malignant condition that provides a window of opportunity for intervention with either curative resection or ablation therapy. [Shaheen et al., Am. J. Gastroenterol. 111: 30-50 (2016)]. Conventional endoscopic surveillance using white light illumination with random four-quadrant biopsy has been found to have limited effectiveness for localizing dysplasia that is flat in appearance, focal in extent, and patchy in distribution [Shaheen et al., supra]. Chemoprevention with nonsteroidal anti-inflammatory drugs, such as COX-2 inhibitors, has been limited by unacceptable side effects [Abrams, Therap. Adv. Gastroenterol. 1: 7-18 (2008)].

**[0005]** The clinical use of a peptide monomer with topical administration to detect HGD and EAC in vivo with wide-field endoscopy and confocal microendoscopy has been reported previously [Sturm et al., Sci. Transl. Med. 5: 184ra161-184ra161 (2013); Joshi et al., Endoscopy 48: A1-A13 (2016)]. Systemic delivery of the targeting ligand may improve detection of dysplastic sub-surface glands commonly seen after radio-frequency ablation (RFA) [Odze et al., Endoscopy 40: 1008-15 (2008)]. Lectins have been shown to target Barrett's neoplasia ex vivo [Bird-Lieberman et al., Nat. Med. 18: 315-21 (2012). However, these agents are low in diversity and may not have adequate binding affinity for clinical use. An Alexa Fluor 488 labeled monoclonal antibody has been administered systemically in a rat model of BE, and found heterogenous expression of ErbB2 (HER2) in EAC using confocal microendoscopy in vivo [Realdon et al., Dis. Esophagus 28: 394-403 (2015)].

**[0006]** Thus, new products and methods for detection and treatment of EAC, HGD, and Barrett's neoplasia are needed in the art. New products and methods would have important clinical applications for increasing the survival rate for EAC, and for reducing related healthcare costs.

## SUMMARY

**[0007]** The ability to identify target expression can be used to accurately diagnose, stage, and classify tumors, and to monitor their response to therapy. EAC is highly heterogeneous on gene expression profiles [Dulak et al., Cancer Res. 72: 4383-93 (2012)]. Epidermal growth factor receptor (EGFR) and epidermal growth factor receptor 2 (ErbB2) have been found to be high-frequency gene amplified and overexpressed in HGD and EAC [Dahlberg et al., Ann. Thorac. Surg. 78: 1790-1800 (2004); Cronin et al., Am. J. Gastroenterol. 106: 46-56 (2011)]. These receptor tyrosine kinases are validated cancer biomarkers and function to stimulate epithelial cell growth, proliferation, and differentiation [Citri et al., Nat. Rev. Mol. Cell Biol. 7: 505-16 (2006)]. Emerging evidence supports early expression of EGFR and ErbB2 in progression of BE to EAC when intervention can improve patient outcomes [Paterson et al., J. Pathol. 230: 118-28 (2013)]. It is contemplated herein that their location on the cell surface is well suited for development as either a diagnostic or therapeutic target. Minimal overlap in expression has been found in studies of surgically resected EAC specimens [Miller et al., Clin. Cancer Res. 9: 4819-25 (2003)]. A multiplexed approach that detects these two targets in combination is contemplated herein.

**[0008]** Multivalent ligands generate synergistic effects that can increase their affinity, avidity, selectivity, and potency by binding multiple targets concurrently [Rao et al., Science 280(5364): 708-11, 1998]. These properties can enhance in vivo diagnostic imaging performance by improving target-to-background (T/B) ratio and detecting targets at lower levels of expression [Luo et al., Mol. Pharm. 11: 1750-61 (2014)]. Also, this strategy can achieve additive effects for therapy by interfering with interconnected cell signaling pathways [Cochran, Sci. Transl. Med. 2: 17ps5-15, 2010]. The ability to bind multiple targets simultaneously may reduce acquired resistance that arises from prolonged binding a single target [Rosenzweig, Biochem. Pharmacol. 83: 1041-8 (2012)]. A number of bispecific antibodies have been developed for dual targeting, however effectiveness may be limited by poor tumor uptake, immunogenicity, and high manufacture costs [Wu et al., Nat. Biotechnol. 23: 1137-46 (2005)]. Seven amino acid peptide monomers specific for EGFR and ErbB2 have been validated [Zhou et al., Clin. Transl. Gastroenterol., 6(7): e101 (2015); Joshi et al., Bioconjug. Chem. 27:481-94, (2015)]. The disclosure herein combines these EGFR and ErbB2 peptide monomers in a heterodimer configuration to provide a novel and effective strategy for improved targeting performance.

**[0009]** The disclosure provides a reagent comprising a heterodimeric peptide comprising an epidermal growth factor receptor (EGFR)-specific peptide and an epidermal receptor growth factor 2 (ErbB2)-specific peptide, or a multimeric form of the heterodimeric peptide, wherein the heterodimeric peptide specifically binds to EGFR and ErbB2, and at least one detectable label, or at least one therapeutic moiety, or both, wherein the label, the therapeu-